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Identification of Peyote via Major Non-Phenolic Peyote Alkaloids

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A simple method is used to extract the major non-phenolic peyote alkaloids. A portion of the extract is subjected to a presumptive chemical test found to be specific to date for peyote among cacti. The remaining extract is used, as desired, for one of three complementary methods. The first method is adequate in the absence of reference alkaloids for identification of 0·5g or more of peyote material by TLC, color tests and UV spectra. The second method is adequate in the absence of reference alkaloids for identification of 2g or more of peyote material via TLC and IR spectra. The third method is primarily used in conjunction with a previously "standardized" extract for identification of 50mg or more of peyote plant material.

Introduction

The forensic identification of peyote (*Lophophora williamsii*) can be a problem in the absence of an intact button. If the peyote is dried and pulverized, even a trained botanist may be unable to perform this identification. In the absence of suitable morphological characteristics, the criminalist should be able to identify peyote on the basis of the major peyote alkaloids. Most criminalists identify peyote by morphology in combination with extraction, isolation and identification of Mescaline. Mescaline is known to be present in at least 11 other species of cacti. Table 1 lists the cacti which contain Mescaline and other non-phenolic alkaloids (Agurell, 1969, 1971; Kapadia, 1968, 1970). Examination should include an additional identification of the major alkaloids of peyote such as Anhalonine and Lophophorine. The chemical structures of these alkaloids are illustrated in Figure 1. The numerous papers on *TLC* methods of peyote identification fail to take into account that most forensic laboratories lack pure drug standards of the major peyote alkaloids other than Mescaline. For this reason, this paper deals with the certification of a uniformly produced extract of the major non-phenolic alkaloids and the subsequent use of such a reference extract in routine work.

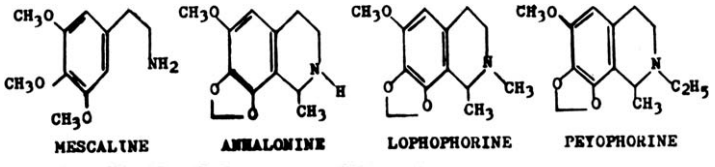

Fig. 1. The chemical structure of the major non-phenolic alkaloids.

Methods

1. Extraction

Moderately pulverize 0·5g or more of dried "peyote". If more than 0·5g peyote is used, the heating time, digestant, wash and extractant volumes must all be proportionately increased to avoid filtration and emulsification difficulties. If green peyote is used in this procedure, it is recommended that at least 1g of green peyote material be used for extraction. Add 15ml 10% HCl to the pulverized material in an Ehrlenmeyer flask and immerse the flask in a boiling

water bath for 15 minutes. Remove the flask from the boiling water bath and filter through Whatman No. 1 filter paper. After the first filtrate is obtained add 10ml more distilled water to the filter paper as a wash. Adjust the filtrate to pH 11 or higher with conc. NaOH solution. Extract the filtrate with 50ml diethyl ether. A second extraction of the mother liquid is unnecessary. If emulsification occurs in extraction, the emulsion can readily be broken by centrifugation. Since the emulsion contains ether, a closed centrifugation container is recommended. Evaporate the diethyl ether extract to 10ml.

TABLE 1

NON-PHENOLIC ALKALOIDS OF CACTI CONTAINING MESCALINE

Cacti	*Non-Phenolic Alkaloids*
Lophophora williamsii	Mescaline
	Anhalonine
	Anhalinine
	Lophophorine
	N-Methylmescaline
	N-Acetylmescaline
	3,4-Dimethoxyphenylamine
	O-methylanhalonidine
	Peyophorine
Trichocereus bridgesii	Mescaline
	3,4-Dimethoxyphenylethylamine
Trichocereus cuzcoensis	Mescaline
Trichocereus fulvilanus	Mescaline
Trichocereus macrogonus	Mescaline
	3,4-Dimethoxyphenylethylamine
Trichocereus pachanoi	Mescaline
	3,4-Dimethoxyphenylethylamine
Trichocereus pascana	Mescaline
Trichocereus schickendantzii	Mescaline
Trichocereus taquimbalensis	Mescaline
	3,4-Dimethoxyphenylethylamine
Trichocereus terscheckii	Trichocereine
	Mescaline
Trichocereus validus	Mescaline
Trichocereus werdermannianus	Mescaline
	3,4-Dimethoxyphenylethylamine

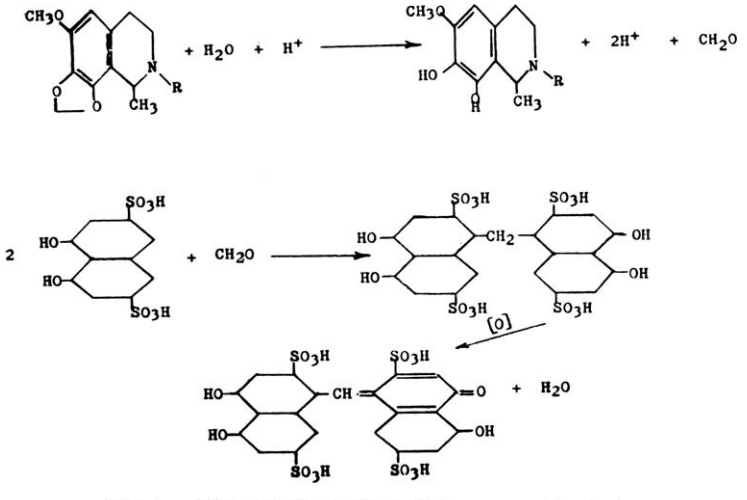

Fig. 2. The overall reactions of the presumptive test.

2. Chromotropic Acid Presumptive Test

According to the present literature on cactus alkaloids the only alkaloids possessing the methylenedioxy group are Anhalonine, Lophophorine and Peyophorine which are unique to Peyote. The chromotropic acid test is specific for formaldehyde which is released by decomposition of the methylenedioxy group in hot concentrated H_2SO_4. The formaldehyde forms a condensation reaction with chromotropic acid. A red-violet chromaphore is formed by oxidation (Feigl, 1956). The mechanism of the decomposition of the methylenedioxy group in hot concentrated H_2SO_4 to form formaldehyde is not fully understood. The overall reaction appears in Figure 2. The presumptive test is performed by placing approximately one-tenth of the ether extract obtained above in a test tube and exaporating to dryness. Approximately 50mg of chromotropic acid is added to the test tube followed by 3ml conc. H_2SO_4. Immerse the tube in a boiling water bath for 2–4 minutes. A purple-red color indicates the presence of compounds bearing the methylenedioxy group such as in the case of Peyote–Anhalonine, Lophophorine and Peyophorine. Table 2 lists the reactions obtained with extracts of the cacti tested.

TABLE 2

RESULTS OF CHROMOTROPIC ACID TEST AND *TLC* ON SEVERAL PURPORTED MESCALINE-CONTAINING CACTI AND OTHERS

Cactus	*Chromotropic Acid Test*	*Mescaline**
Lophophora williamsii	Strong reaction within 2–4 minutes	Positive
Trichocereus bridgesii	Negative	Positive
Trichocereus pacanoi	Negative	Positive
Trichocereus pascana	Negative	Negative
Trichocereus macrogonus	Negative	Positive
Trichocereus grandiflorus	Negative	Possible trace
Trichocereus terezensis	Negative	Negative
Trichocereus spachianus	Negative	Negative
Trichocereus peruvianus	Negative	—
Carnegia gigantea	Negative	Possible trace
Pelecyphora aselliformis	Weak reaction within 20–30 minutes	Negative

The following were negative in the chromotropic acid test and negative for mescaline: *Gymnocalycium gibbosum, Gymnocalycium andreae, Gymnocalycium hossei, Gymnocalycium monvillei, Gymnocalycium zegarras, Gymnocalycium quehlianum, Gymnocalycium deesianum, Gymnocalycium denudatum, Gymnocalycium verturianum, Coryphantha dainiocereus.*

**As indicated in routine TLC procedure by reaction with ninhydrin and Rf.*

3. TLC Procedures

Procedure A: TLC combined with color tests and UV spectra. This procedure requires at least 0·5g dried peyote material. Spot the ether extract in a narrow streak approximately 5 inches long on a glass *TLC* plate having a coating of Silica Gel G at least 250μ thick. Run the plate in the system chloroform-butyl alcohol: conc. NH_4OH (50:50:2·5) (Lundstrom, 1967) (Eastman or similarly prepared sheets with an organic binder cannot be used with this solvent system). After elution, the removal of NH_3 is necessary to avoid interference with the spray reactions. The residual NH_3 is removed, by carefully heating the *TLC* plate in an oven. Excessive heating is to be avoided since it will produce loss of the alkaloids. After the NH_3 is removed cover the plate and expose approximately ½ inch at each end of the streak. Spray each exposed end first with ninhydrin reagent. Ninhydrin spray will visualize the Mescaline within 3–5 minutes.

After 5 minutes, again blocking off thc plate as described above, spray with the Iodoplatinate-Dragendorff reagent or acidified iodoplatinate. Iodoplatinate-Dragendorff reagent is superior to plain or acidified iodoplatinate sprays. The pray reagent formulae are at the end of this paper.

After the above spray procedure, the visualized alkaloid bands on the end portions of the *TLC* plate are used to locate the non-phenolic alkaloids in the unsprayed center portion of the *TLC* plate. Table 3 lists the Rf values of the alkaloids.

Chemical color tests are run on scrapings from these alkaloid bands and scraping from blank areas above the solvent front and below the spotting area. The control above the solvent front will indicate the color was not caused by the silica gel. The control below the spotting area will indicate the color was not caused by solvent.

The color test reagents used are Marquis, Mecke, Froehde, Mandelin and Concentrated Nitric acid. The color results are listed in Table 4.

The *UV* maxima are obtained by placing the remaining scrapings in centrifuge tubes containing 3·5ml 0·1N HCl and centrifuge. Transfer the centrifugating into a cuvette and scan the solution in the *UV* from 320 to 220nm. The *UV* maxima are as follows: Mescaline 269nm, Anhalonine 276nm and Lophophorine 274·5nm.

TABLE 3

Rf VALUES OF THE MAJOR NON-PHENOLIC PEYOTE ALKALOIDS IN SYSTEM CHLOROFORM: N-BUTYL ALCOHOL: Conc. NH_4OH (50:50:2·5)

Alkaloids	*Rf*	*Ninhydrin*	*Iodoplatinate-Dragendorff*
Mescaline	0·46	Purple	Brownish-purple
Anhalonine	0·66	No reaction	Brownish-purple
Lophophorine	0·89	No reaction	Brownish-purple
Yellow *UV* Fluorescence	0·94	No reaction	Grayish-blue
Peyophorine	0·96	No reaction	Brownish-purple

TABLE 4

COLOR TEST OF MAJOR NON-PHENOLIC PEYOTE ALKALOIDS

Test	*Mescaline*	*Anhalonine*	*Lophophorine*
Marquis	Orange	Lavender	Lavender
Mecke	Yellow-orange	Green (1 min) to blue	Green (1 min) to blue
Frohde	Brown	Yellow green (15 sec) to gray (10 sec) to black (1 min) black violet	Yellow green (15 sec) gray (10 sec) black (1 min) black violet
Mandelin	Brown	Blue	Blue
Conc. HNO_3	Red	Reddish-pink	Reddish-pink

Procedure B: TLC combined with IR spectra. This following alternative is purely *TLC-IR* method of standardization and may be preferred by some workers. At least 2g peyote material is needed for this approach. Use the same *TLC* solvent system and spraying procedure as described in Procedure A. Scrape off the unsprayed silica gel areas containing the presumed Mescaline, Anhalonine and Lophophorine and transfer the material from each of the alkaloid bands to individual Pasteur pipets with small wads of glass wool in the necks. Elute the material in each pipet with 2–3ml of $CHCl_3$: MeOH: conc. NH_3OH (80:20:1). Evaporate the eluates to dryness on steam bath; immediately remove from steam bath when dry, dissolve in small quantity of $CHCl_3$ and coat silver chloride disks drop-wise with the solutions. Figures 3, 4, 5 show the *IR* spectra obtained as described above.

Procedure C: TLC utilizing a reference standard. Eastman Chromagram 6061 Silica Gel sheets are cut to needed width and length (5–6 inches is convenient). Care must be taken to cut both sides of the strip with the same technique or slanted fronts will result during the elution. After the chromotropic acid test, the residual ether extract is spotted side by side in two approximately $\frac{1}{2}$ inch streaks. The second of these streaks is overlayed with the reference peyote extract, providing in effect an "internal standard" in this method. The reference extract is applied alone in a third streak. The sheet is run in ethylene dichloride saturated with ammonia (25ml ethylene dichloride is shaken vigorously with 1ml concentrated ammonium hydroxide. After 1 minute of standing

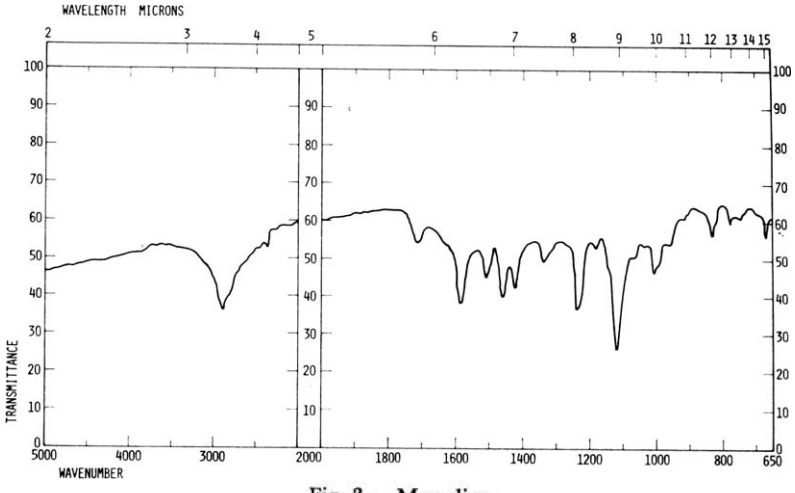

Fig. 3. Mescaline.

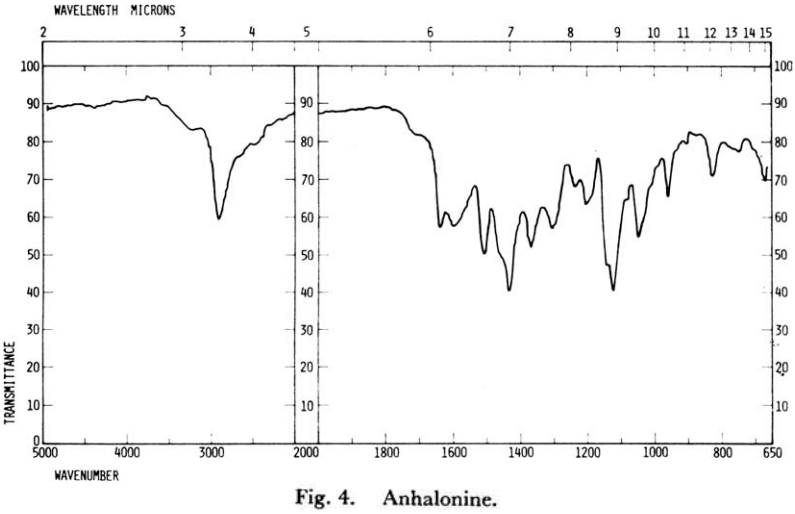

Fig. 4. Anhalonine.

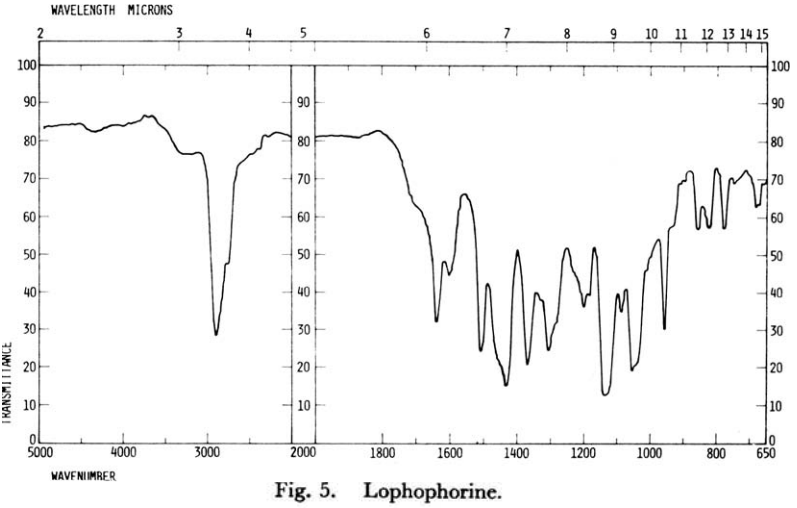

Fig. 5. Lophophorine.

in the separator funnel, the organic phase is separated and the organic phase is used). Allow the solvent to run up approximately 4/5th of the height of the sheet. The sheet is dried in a hot air draft and heated in an oven until warm. The sheet is then inspected under short-wave *UV* light for concordance in the three eluted bands of the strongest bands of yellow and blue fluorescence. The same spray procedure is used as for the previous procedure over the entire sheet. The Rf values are listed in Table 5.

TABLE 5

Rf VALUES OF THE MAJOR NON-PHENOLIC PEYOTE ALKALOIDS IN *TLC* SYSTEM OF PROCEDURE C

Alkaloids	*Rf*	*Ninhydrin*	*Iodoplatinate-Dragendorff*
Mescaline	0·13	Purple	Brownish-purple
Anhalonine	0·42	No reaction	Brownish-purple
Yellow *UV* fluorescence	0·55	No reaction	Grayish-blue
Lophophorine	0·66	No reaction	Brownish-purple
Peyophorine	0·78	No reaction	Brownish-purple

Discussion

The simple extraction procedure avoids equipment such as Soxhlet extractors, reflux condensors, ion-exchange resin columns as well as "overnight" extraction. It is suitable for large scale concurrent extraction of a number of samples. It is desirable to accumulate a substantial quantity of extract for reference purposes for future use in routine identification.

A number of cacti were subjected to the chromotropic acid presumptive test. Table 2 is the summary of the chromotropic acid tests. We have yet to find one yielding a non-phenolic alkaloidal extract giving a positive test other than peyote. The nearest to a positive reaction was given by the cactus *Pelecyphora aselliformis* but the reaction was both weak and considerably delayed beyond the specifications of the test. A color reaction in 2–4 minutes in the chromotropic acid is considered a positive test; however, this cactus extract gave a weak color reaction in 20 minutes. This cactus, coincidentally, is called "Peyote" by the Indians of Mexico.

The *TLC* procedure A or B is intended for rigorous certification, in absence of pure drug standards, of a non-phenolic peyote alkaloid extract. Procedure A which involves color tests and ultraviolet absorption of the three major peyote non-phenolic alkaloids required at least 0·5g of dried peyote. Procedure B which employs *IR* spectral methods for identifying the three major Peyote alkaloids required at least 2g of dried Peyote. The authors consider either procedure adequate for the forensic identification of Peyote.

After following through Procedure A or B, it is recommended that the unused extract be retained as future *TLC* reference standard, avoiding repeated standardization. It is found that these alkaloids as free bases stored dry at room temperature will decay with time. Preserving the alkaloidal extract in methanol and keeping it under refrigeration will slow this decaying process. The yellow and blue *UV* fluorescing materials tend to decay with time of storage faster than the rest of the alkaloids.

For future peyote identification using this standardized extract proceed to Procedure C. The advantages of this *TLC* procedure over the preceding methods are convenience of filing of the Eastman sheets, ease of removal of ammonia and separation of the yellow fluorescing unknown substance from peyophorine. The disadvantages are ease of overloading and heading of the Mescaline streak. By omitting the chromotropic acid test and applying the extract in spots rather than streaks on the Eastman sheet, it is possible to identify with as little as 50mg of dried or 250mg green peyote material.

Reagent formulae

Iodoplatinate Spray Reagent. Dissolve 0·25g of platinic chloride and 5g of KI in sufficient water to make 100ml.

Modified Dragendorff Spray Reagent. Solution A. 0·85g bismuth subnitrate, 10ml glacial acetic acid and 40ml water. Solution B. KI, 40g, 100ml water.

The reagent is prepared by mixing 5ml of solution A with 5ml solution B, 20ml glacial acetic acid and 100ml water.

Iodoplatinate-Dragendorff Spray Reagent. This spray is made by mixing equal parts of the iodoplatinate and the modified Dragendorff reagents.

Ninhydrin Spray Reagent. Dissolve 50mg ninhydrin in 100ml acetone.

Froehde's Reagent. Dissolve 50mg of molybdic acid or sodium molybdate in 10ml hot concentrated sulfuric acid. The solution should be colorless.

Marquis' Reagent. Add 8–10 drops of 40% formaldehyde solution to 10ml pure concentrated sulfuric acid.

Mandelin's Reagent. Dissolve 1g ammonium vanadate in 100ml concentrated sulfuric acid.

Mecke's Reagent. Dissolve 0·25g selenious acid in 25ml concentrated sulfuric acid.

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